# Genetics of Disease (PRRS and PCVAD) Resistance

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**Introduction.** Pigs exhibit a range of responses when exposed to certain pathogens. Some die, some become severely ill and never fully recover, others become moderately ill and quickly recover, and some show few symptoms of disease. Evidence is accumulating that part of this variation is due to variation among pigs in their immune function genes. For some diseases, immunity is acquired – pigs produce antibodies after exposure to a pathogen or vaccine. Pigs may vary in how rapidly and effectively their immune system develops antibodies to cope with this foreign material. For other pathogens, some pigs may have natural or innate immunity. But all pigs are not alike; some have greater innate protection than others, resulting in the range of responses described above.

Whether selection for disease resistance should be implemented depends on the disease, the magnitude of genetic variation in the host (the pig), and the economic efficacy of vaccination/management practices to control it. For many diseases, there are very cost-effective vaccines or management practices to control them and there is little reason to attempt to select for greater immunity. For others, vaccines may be only partially effective or the pathogen changes so quickly that the outcome of vaccination is uncertain. If these diseases are widespread and cause severe economic losses, genetic selection to enhance resistance may be warranted.

The ideal situation occurs when we can select directly on genotypes for genes coding resistance/susceptibility. For example, susceptibility to colonization by F18 bearing *E. coli* is controlled by a dominant allele and resistance by a recessive allele (Bertschinger et al., 1993) and a PCR-RFLP test for the mutation in the encoding gene is available (Meijerink et al., 1997). Therefore, selection for resistance to F18 E. *coli* can be practiced without exposure to the bacteria. Unfortunately, genetic markers for genes involved in immune responses to most diseases do not exist. Furthermore, selection for resistance to such diseases using quantitative methods is generally not feasible. Such selection requires that each generation of a population must be raised in the presence of the disease and those pigs displaying the least symptoms selected as breeders. Uniform quantification of health in breeding populations is difficult and little is known about the relative magnitude of genetic and environmental variation for most diseases. Furthermore, such a management strategy would be devastating to seedstock breeders. They would have very few customers if their breeding stock carried pathogens that automatically transferred disease to their customers, even if over time, the severity of the disease diminished because of the selection practice in the breeding herd.

Therefore, we must develop strategies to select for resistance to diseases in nucleus breeding populations that are maintained with the strictest of management strategies to maintain high health status. Identifying genes involved in immunity, markers for these genes, or proteins produced by these genes that accurately predict the outcome when pigs are exposed to pathogens are critical for success. First, traits (phenotypes) that measure variation among pigs in response to pathogens must be defined. Then, the genes responsible for this variation can be identified.

At Nebraska, we are using such an approach to study genetic variation in pigs for two of the most serious diseases facing swine producers today, Porcine Reproductive and Respiratory Syndrome, caused by the PRRS virus (PRRSV), and Porcine Circovirus Associated Disease

(PCVAD), caused by Porcine Circovirus Type 2 (PCV2). PCV2 is considered to be the agent responsible for Postweaning Multisystemic Wasting Syndrome, although other pathogens also are involved in expression of PCVAD. Experiments are underway to determine whether genetic variation in pigs exists for these diseases and to develop strategies to select for greater resistance. The purpose of this report is to provide an update on progress toward achieving these goals.

***PRRS.*** A PRRSV infection experiment was initiated in 2003 with 400 pigs, including 200 pigs from each of the NE Index line (I), a Large White-Landrace composite population that has been selected for increased litter size for 20 generations, and 200 pigs from a cross of Hampshire and Duroc lines (HD) that have been selected for rate and efficiency of lean growth. Line I pigs were born in the University of Nebraska swine research herd, whereas HD pigs were obtained from a commercial farm. Neither farm had experienced PRRS; before initiation of the experiment, pigs from both herds had tested negative for PRRSV. Pigs were selected at random from available litters and included two pigs of the same sex representing a total of 83 sires and 163 dams.

Pigs were transported from their farm of origin at an average age of 23 d to the University of Nebraska Veterinary and Biomedical Sciences (VBS) Animal Research Facility and placed in environmentally controlled rooms with 25 pigs per room. Each room contained one pen of pigs of each line with 12 to 13 pigs per pen. Within each replication, one room was randomly assigned for infection; pigs in the other isolated room, which were littermates to those in the infected room, served as controls. After a 3-d acclimation period, pigs in rooms designated for infection were inoculated with a 2 ml dose of $10^5$ $CCID_{50}$ of PRRSV strain 97-7985, a standard virus used in the PRRS research of Dr. F. Osorio at UNL.

*Traits Measured.* Based on the temporal sequence of events upon infection of a pig with PRRSV described by Osorio (2002), days 0, 4, 7 and 14 were selected as data collection points to capture the maximum amount of viral replication in the pigs. Body temperature by rectal probe, body weight, and blood draws of all pigs were collected and recorded just before inoculation (d0) and 4, 7, and 14 d after inoculation. On d14, all pigs were sacrificed, necropsy was performed, and samples of lung, bronchial lymph node, and spleen were collected and stored. Level of viremia, the pig's ability to replicate virus, was measured in serum, lung, and bronchial lymph node tissue of each pig. Serum samples collected at d14 from pigs infected with PRRSV and their uninfected littermates were analyzed with an ELISA® to determine the level of PPRSV antibody and to test for possible cross contamination in uninfected pigs.

Lungs were scored for the presence of pneumonia (yes (1) or no (0)). Representative sections of the left cardiac lobe of the lung of each pig were processed and scored for lesions while being examined by light microscopy. Scores ranged from 1, no lesions, to 3, lung sections that had greater than 50% involvement of severe interstitial pneumonia.

*Biological results*. Pigs infected with PRRSV gained less weight ($P < 0.001$) during each interval than their uninfected littermates (Figure 1), but the pattern of response was different between lines (line x treatment and line x treatment x interval, $P < 0.001$). Uninfected HD pigs gained weight more rapidly from d4 to d7 ($0.06 \pm 0.08$ kg) and from d7 to d14 ($0.66 \pm 0.08$ kg) than I pigs. However, I pigs infected with PRRSV gained more from d4 to d7 ($0.14 \pm 0.08$ kg) and from d7 to d14 ($0.32 \pm 0.08$ kg) than infected HD pigs.

Rectal temperature (Figure 2) was affected by line, treatment, and interactions (line, treatment, line x treatment, and line x treatment x day; $P < 0.001$). Infected pigs of each genetic line had higher rectal temperatures at d4, d7, and d14 than their uninfected littermates. Overall, HD pigs had higher temperature than I pigs ($P < 0.001$) and mean temperature increased in both

lines from d0 to d14 (P < 0.001). The pattern of response for uninfected pigs was similar in both lines; however the response in infected littermates was different. Temperatures increased more rapidly in infected HD pigs, especially from d0 to d4, and remained higher to d14. Rectal temperatures of infected pigs of both lines seemed to peak at d7 (39.40º C for I and 40.15º C for HD) and then decrease slightly at d14 (39.34º C for I and 39.88º C for HD).

Figure 1. Weight change from day 0 to 4, d 4 to 7, and d 7 to 14 for Index (I) and Hampshire-Duroc cross (HD) pigs without (-) and with (+) PRRSV infection (treatment, line x treatment, and line x treatment x interval: P < 0.001)

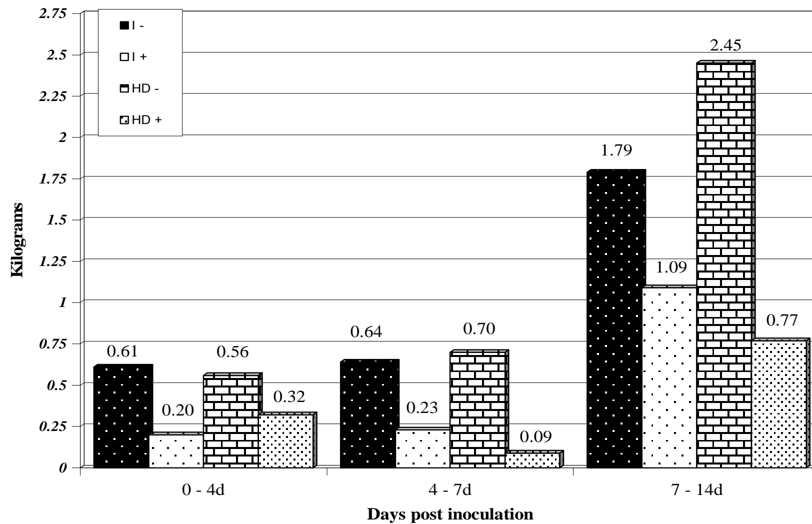
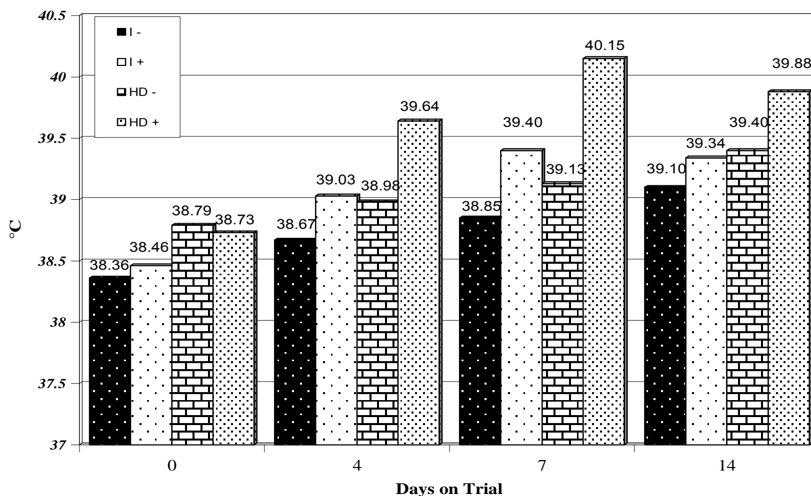


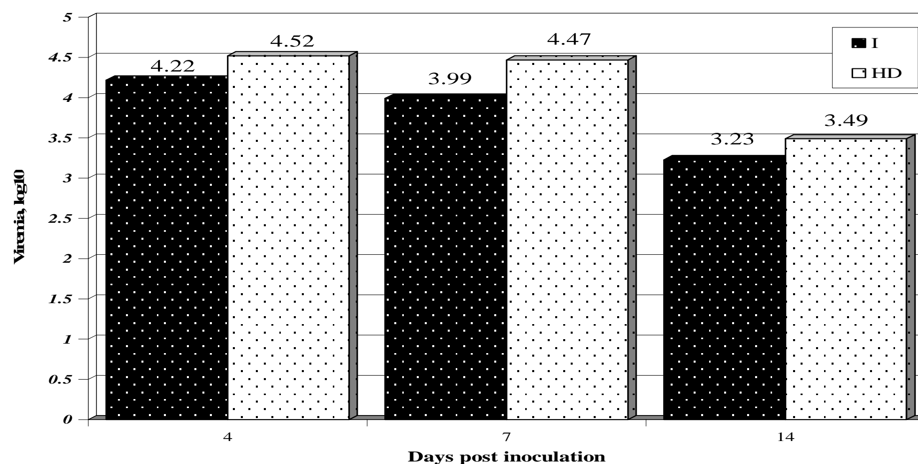

Figure 2. Rectal temperature (°C) at day 0, 4, 7, and 14 post-infection for uninfected (-) and infected (+) Index (I) and Hampshire-Duroc (HD) pigs (line, treatment, line x treatment, line x treatment x day; P < 0.001)




Mean viremia level is illustrated in Figure 3. Viremia could be recorded only in blood drawn from infected pigs at d4, d7, and d14. Values in the graph are base 10 logarithms, so differences in exponents represent exponentially greater differences in number of $CCID_{50}$/ml.

For example, the coefficients of 4.22 and 4.52 for I and HD pigs at d4, respectively, represent a two-fold difference in number of $CCID_{50}$/ml of blood. Viral titer level was greater in HD than I pigs at d4, d7, and d14 ($P < 0.001$); line x day interaction was not important ($P > 0.30$). Differences between lines in viral load in lung tissue and bronchial lymph nodes were similar to that in serum. HD pigs tended to have higher PRRSV titer in the lung ($0.41 \pm 0.14$, $P = 0.11$) and the bronchial lymph nodes ($0.51 \pm 0.14$, $P = 0.07$) than I pigs.

Figure 3. Viremia titer, expressed as $\log_{10}$ $CCID_{50}$/ml, in serum of Index (I) and Hampshire-Duroc cross (HD) pigs at 4, 7, and 14 d post-infection with PRRSV (line, $P < 0.0001$; SEM = 0.07).

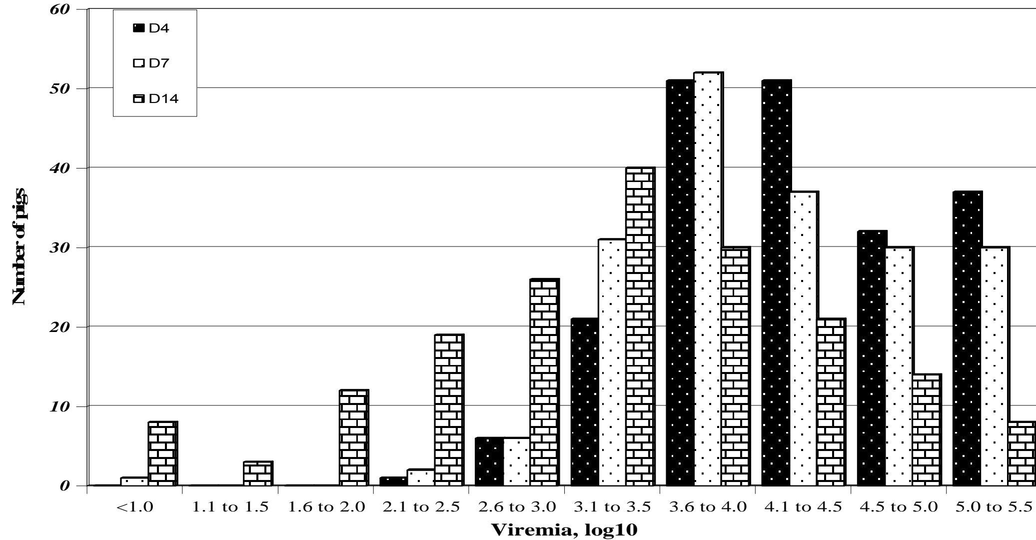



Line differences and line by treatment interactions across days are evidence that genetic variation in the mechanisms involved in immune responses to PRRSV exist and provided impetus for the next phase of the study. Figure 4 illustrates the variation across all pigs in viremia (viral load). All pigs were replicating virus at d4, but replication rates varied greatly. Pigs in the right tail of the distribution replicated the virus at very high rates, as high as $10^{5.5}$ (316,228) $CCID_{50}$/ml, whereas those in the other tail had replication rates as low as $10^{0.7}$ (5) $CCID_{50}$/ml of blood. High levels of viral load tended to be associated with low weight gain, high rectal temperature, and increased incidence of lung lesions, but correlations among these variables were low (absolute values of correlations ranged from 0 to 0.46). Some pigs replicated the virus at high rates and showed all the clinical symptoms of PRRSV. They grew slowly, had high body temperature, and had gross and microscopic lung lesions typical of PRRSV infection. Other pigs with similar levels of viremia showed few symptoms of PRRS. They gained weight at normal rates, had normal or only slightly elevated body temperature, and had few lung lesions. Similarly, there were pigs in this sample with relatively low levels of viral load that showed typical symptoms of PRRSV; whereas, others had few clinical signs suggestive of PRRSV infection.

The challenge is to find the genes and/or gene products responsible for this variation. The first step in this process was to use stored tissues to characterize the genetic differences observed in the PRRSV-infection experiment. Lung and bronchial lymph tissue were used to determine differences in expression of specific immune function genes and levels of cytokines, proteins produced by immune function genes, between pigs classed as more resistant and more susceptible to PRRSV infection. It was accomplished by doing a gene expression study with a

sub-sample of 56 of the 400 pigs in the experiment. These 56 pigs were the 7 most resistant and 7 most susceptible in each line, and their uninfected littermates.

Figure 4. Distribution of viremia, $log_{10}$, at d4, d7, and d14in serum of infected




pigs.

*Immune Gene Expression Experiment*. Twenty eight infected pigs and their littermates were chosen for study of expression differences for specific immune function genes on the basis of a Principal Component Index. This index captured all the variation among all traits recorded and separated pigs on a continuous scale from most resistant to most susceptible. High (H) indexing pigs were most susceptible and low (L) indexing pigs most resistant to PRRSV. Means for the seven H and seven L pigs of each line are in Table 1. Responses are similar, but more dramatic, than average responses for all pigs. Pigs in the H-class of both lines had high serum viremia levels at each day, indicating high disease burden to the end of the evaluation period. Line I pigs in the L class, however, began clearing the virus by d7 and had relatively low levels at d14. The HD, H-class pigs responded somewhat differently, having greatest levels of serum viremia at d7, declining at d14, but not reaching the same low values of L-class, I pigs.

Responses in weight gain were consistent with responses in viremia. The H-class pigs either lost weight or gained very little weight whereas both I and HD L-class pigs gained weight during each period.

*Gene expression analyses.* Eleven specific immune genes involved in both innate and acquired immunity were evaluated. Polymerase Chain Reaction (PCR) was used to amplify the number of copies of each gene. The resulting Ct value then relates directly to the amount of PCR product and therefore to the initial amount of target DNA present in the sample. Samples producing high Ct values had less cDNA than samples producing low values because more PCR cycles were needed to reach the threshold. Cytokine protein levels in serum were also measured. Therefore, the experiment evaluated gene expression differences and cytokine differences in a 2x2x2 factorial design (2 genetic lines - I & HD, susceptible and resistant classes – H vs. L, and infected (+) and uninfected (-) littermates).

Table 1. Overall line means (μ) and means for the seven high (H) and low (L) responders for Index (I) and Hampshire-Duroc (HD) cross pigs infected with PRRSV.

| | Line I | | | Line HD | | |
|---|---|---|---|---|---|---|
| | M | H | L | μ | H | L |
| V4 | 4.17 | 4.39 | 4.11 | 4.54 | 5.11 | 3.10 |
| V7 | 3.91 | 4.47 | 3.20 | 4.40 | 5.13 | 3.64 |
| V14 | 3.00 | 4.49 | 0.50 | 3.59 | 5.29 | 2.51 |
| $WC_{0-4}$ | 0.32 | 0.08 | 0.58 | 0.29 | -0.04 | 0.53 |
| $WC_{4-7}$ | 0.33 | -0.01 | 0.92 | 0.06 | -0.18 | 0.37 |
| $WC_{7-14}$ | 1.35 | 0.31 | 2.21 | 0.71 | -0.57 | 1.80 |
| $TC_{0-4}$ | 0.92 | 1.09 | 0.33 | 1.76 | 0.79 | 1.94 |
| $TC_{4-7}$ | 0.58 | 1.00 | 0.66 | 0.82 | 1.36 | -0.06 |
| $TC_{7-14}$ | -0.16 | 0.36 | -1.01 | -0.36 | -2.84 | -0.26 |
| LV | 3.96 | 5.07 | 2.40 | 4.45 | 4.71 | 4.21 |
| LNV | 2.55 | 3.33 | 1.31 | 3.12 | 3.70 | 2.66 |
| L | 1.26 | 1.57 | 1.00 | 1.96 | 1.57 | 2.00 |

[1]V4, V7, and V14 = viremia titer, expressed as $\log_{10}$ $CCID_{50}$/mL, (cell culture infectious dose 50%/mL) in serum collected at 4, 7, and 14 d, respectively; $WC_{0-4}$, $WC_{4-7}$, and $WC_{7-14}$ = weight change, kg, from d0 to d4, d4 to d7, and d7 to d14, respectively; $TC_{0-4}$, $TC_{4-7}$, and $TC_{7-14}$ = temperature change, ºC, from d0 to d4, d4 to d7, and d7 to d14, respectively; LV and LNV= viral titer, $\log_{10}$ $CCID_{50}$/mL, in lung and bronchial lymph, respectively, collected at necropsy on d14; L = severity score of lung lesions.

One objective was to determine whether response to infection was a general characteristic of H and L class pigs or whether expression differences could be measured only in the presence of virus. From a genetic selection standpoint, it would be desirable to select on a trait in uninfected pigs that is correlated with response in infected pigs. Thus, expression of genes in uninfected littermates to H and L class pigs was evaluated.

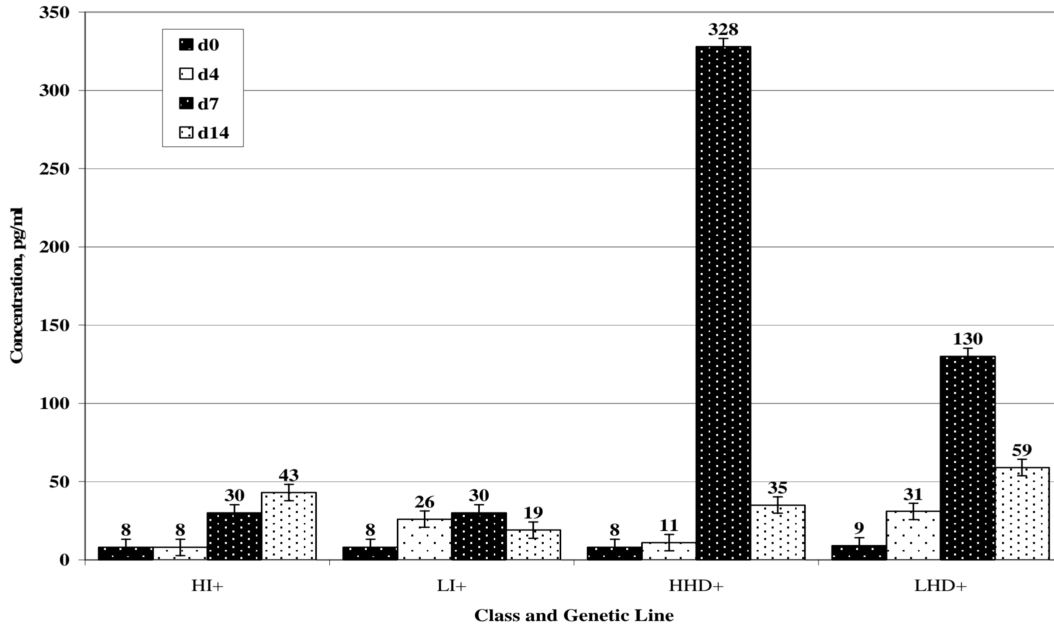
Expression in lung of three genes, Interferon gamma (INFG), Signal transducer and activator of transcription1 (STAT1), and Interleukin 10 (IL10), was greater ($P < 0.05$) in H than L pigs and there was no class by treatment interaction, indicating that the difference was similar in pigs infected with PRRSV and their uninfected littermates. Of the 11 genes studied, these three are candidates for additional research to determine whether their expression in uninfected pigs may be useful in selecting for resistance to PRRSV. Mean Ct values for H and L class pigs were 25.7 vs. 27.5 for INFG, 20.2 vs. 21.4 for STAT1, and 25.0 vs. 27.2 for IL10.

Interaction of class by treatment existed in expression of several other genes. For these genes there was either little difference between uninfected H and L pigs and a significant difference between infected H and L pigs, or the pattern was inconsistent. Therefore, expression of these genes in uninfected pigs will not likely be useful predictors of response to infection.

*Cytokine Protein Levels.* Two cytokines appeared to have value as predictors of response to PRRSV. Levels of the first of these, INFG, are illustrated in Figure 5. Serum of all pigs had minimal amounts of IFNG before infection. The L-class pigs had increased levels of IFNG at d4. Levels were still elevated in both H- and L-class pigs at d7. HD pigs produced substantially greater amounts of IFNG protein than I pigs, especially in the H class at d7. Levels of IFNG at

d14 were lower than at d7, except for infected H-class I pigs for which levels continued to increase over time.

Figure 5. IFNG cytokine protein levels (pg/ml) in serum 0, 4, 7, and 14 days post-infection (d) for high class (H) and low class (L), infected (+) and uninfected (-) Index (I) and Hampshire-Duroc (HD) pigs (day x class x line x treatment: P < 0.01)

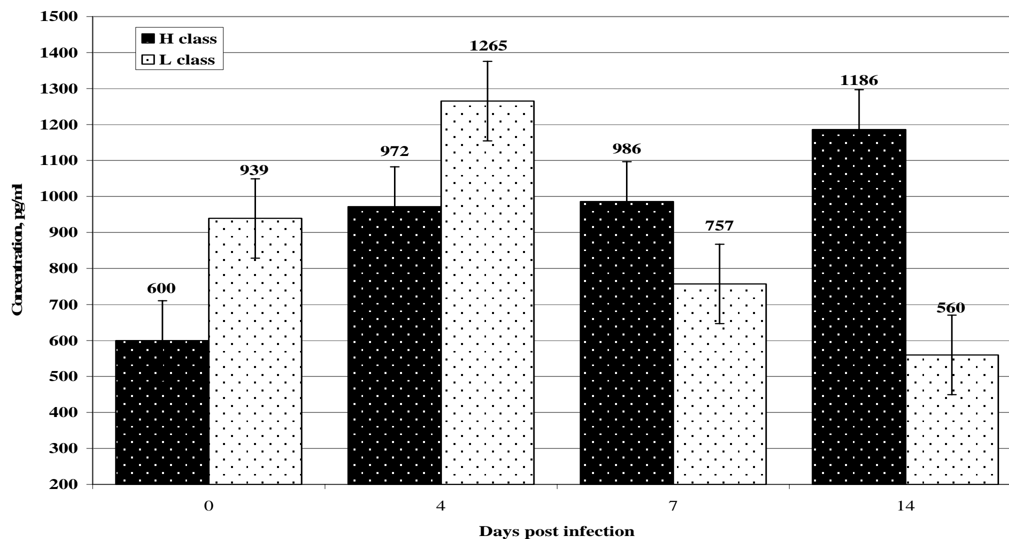



Response for Interleukin 8 (IL8), the other of these cytokines, is illustrated in Figure 6. L-class pigs had 339±114 pg/ml greater levels of IL8 before infection and 293±135 pg/ml higher levels at d4 than H-class pigs. However, the amount of IL8 protein in H-class pigs was 230±159 pg/ml greater at d7 and 626±130 pg/ml greater at d14 than in L-class pigs. Infected pigs had greater levels of IL8 post infection than their uninfected littermates. Pigs in the L-class had an initial increase at d4, but levels returned to normal by d7. The H-class pigs had a continual elevation to d14, indicating they were still responding to the virus.

INFG and IL8 cytokines may be candidates for selection for greater resistance to PRRSV. IL8 appears to be the most useful because high levels in uninfected pigs appear to be associated with resistance. Low levels of INFG in HD pigs were associated with resistance, but not until d7; thus, selection would have to be practiced in pigs infected with virus, making it less useful as a selection trait than IL8.

*Microarray analysis.* DNA from both lung and bronchial lymph tissue of each of the 56 pigs was further hybridized on a microarray on which 13,297 genes had been spotted. This study further confirmed genetic differences in response to PRRSV. Line (I vs. HD) by treatment (infected vs. uninfected) by class (H vs. L responders) interaction existed for expression of five genes in bronchial lymph node (BLN), one gene in lung, and one gene in both tissues; line by treatment interaction existed for expression of one gene in BLN and three genes in lung; treatment by class interaction existed for expression of five genes in lung and one gene in both tissues. Line was significant for 72 genes in BLN, two genes in lung, and 11 genes in both tissues.

Figure 6. IL8 cytokine protein levels (pg/ml) in serum at 0, 4, 7, and 14 d for high class (H) and low class (L) pigs (class x day: P < 0.01)




Many genes respond to infection with PRRSV. Treatment, the difference in expression between infected and uninfected pigs, was significant for 2,645 genes in BLN, 1,513 genes in lung, and 1,502 genes in both tissues. This phase of the experiment revealed possible important genetic associations for fine mapping candidate genes related to response to PRRSV and determining causative alleles.

*Current experiments.* A second PRRSV infection experiment was conducted during fall 2007. It was modeled after the earlier one and was designed to test whether pre-infection levels of IL8 predict response to infection. A total of 230 pigs were infected with the same strain of PRRSV as used in the first experiment. Pigs were weighed and blood samples were drawn at d0 and 4, 7, and 14 d post-infection. Tissue was collected and stored for future use. No results are available as samples are currently being analyzed for viremia and for level of IL8 cytokine.

**PCVAD**. Approximately five years ago, pigs with symptoms of what was then called Postweaning Multisystemic Wasting Syndrome (PMWS, now called Porcine Circovirus Associated Diseases, PCVAD) were observed within the UNL swine herd. Populations of pigs at the farm included the genetic lines being selected for increased litter size and their controls, both of which derived from the same Large White/Landrace composite population, crosses of these lines with industry lines, and crosses of other industry line pigs. Initially it appeared that the condition was occurring only in the Large White/Landrace composite population lines, but not in crossbreds. Furthermore, cursory observations suggested a greater incidence in the selection lines than in the controls, and that the condition was prevalent in some litters whereas no pigs were infected in other litters.

With that background, we implemented a systematic regimen of scoring all pigs of the genetic selection and control lines for symptoms of PCVAD to determine the relative importance of genetic and environmental variation on its expression. A total of 2,554 pigs from Generations 24-26 of two selection lines and two control lines. One selection and control line farrowed in one

season, the other two lines farrowed six months later. The selection lines are selected for increased live pigs per litter followed by within litter selection for rate of lean growth. Control lines are selected randomly. All pigs trace to the same founder boars and gilts born in 1980.

All pigs were managed alike from farrowing through the nursery phase, and again during breeding and gestation. From 60 d of age when pigs were removed from the nursery, they were grown in either completely confined buildings with mechanical heating and ventilation, in modified open-from buildings with thermostatically controlled curtains over side windows but no other mechanical heating or ventilation, or in outside lots containing straw-bedded hoop structures. Pigs were scored every 7-10 days from 70 to 180 days of age for symptoms of PCVAD. Scoring was on a scale of 0 (no symptoms), 1 (suspect), or 2 (positive) for degree of muscle wasting, growth retardation, rough hair coat, and diarrhea.

Only pigs with a score of 2 were considered to be positive for PCVAD. Several pigs moved back and forth between scores of 0 and 1, but never received a score of 2. However, no pig scored as a 2 ever returned to a lower score.

A total of 17.7% of the pigs received a score of 2. A sample of 37 pigs were necropsied and lung, lymph node, tonsil, liver, kidney, thymus, spleen, ileum, and colon tissue were microscopically examined for lesions suggestive of PCVAD. Immunohistochemistry and RT-PCR were used to detect the presence of PCV-2 in these tissues. All 37 pigs were positive for PCV-2. Molecular virology on sub-samples of them determined that the nucleotide sequence was very close to that of PCV2B, a European genotype implicated in outbreaks of severe PCVAD in Eastern Canada and in the US.

PCVAD score was analyzed with ASREML using the Binomial and Probit functions to estimate genetic and environmental effects. Pigs receiving at least one score of 2 were considered positive for PCVAD; pigs scored only as 0 or 1 were considered negative. Direct heritability was low $0.01 \pm 0.001$, indicating little genetic variation among the pigs in whether they expressed PCVAD. Maternal heritability, however, differed significantly from 0 ($0.11 \pm 0.006$), indicating that genes of the dam are partly responsible for whether pigs express PCVAD. The proportions of variance due to common birth litter, common finishing pen, and common year/contemporary group/area were $0.11 \pm 0.032$, $0.05 \pm 0.035$, and $0.06 \pm 0.035$, respectively. Males had a significantly higher probability of PCVAD score than females ($P<0.025$). Significantly greater incidence of PCVAD also occurred in pigs in hoop structures than those in confinement buildings.

Consistent with the maternal genetic effect on expression, pigs scored as positive for PCVAD had significantly lower birth weights (-0.01 kg, $P < 0.05$) than negative pigs, even though pigs seldom received a score of 2 until approximately 90 days of age. This indicates that intrauterine environment plays a role on whether pigs express PCVAD. Positive pigs also weighed significantly less at weaning (-0.52 kg), at 60 d of age (-4.35 kg), and of those that recovered, at 180 d of age (-20.17 kg).

Blood samples were drawn from all pigs at weaning, and at 60, 90, and 120 d of age. Serum from samples drawn at each age from all pigs that were scored as positive for PCVAD and from at least one normal littermate and/or pen-mate were submitted to the Iowa State University Veterinary Diagnostic Laboratory where PCR-PCV2 quantitation and Porcine Circovirus 11 C-ELISA were conducted. Laboratory diagnostics are also performed on samples drawn from dams at farrowing. These same samples are being characterized for other pathogens.

Thus far, diagnostics have been performed on approximately 600 pigs. Analyses of laboratory data have not been performed. However all pigs tested thus far were positive for

PCV2 antibodies at weaning, but negative for viremia at that age. First positive viremia occurred in some pigs at 60 days of age, but was observed most frequently at 90 days. Several pigs were negative for viremia at each age. Thus, the antibodies at weaning are likely maternal antibodies. Scoring and blood draws on current generation pigs will be completed in December, 2007, and samples from another 350 pigs will be submitted for analyses. When these data are collected, genetic analyses of PCV2 viremia and antibody data will be conducted and related to scores. Tissues from all pigs have been stored as a source of DNA and will be used in genetic analyses to identify genes involved in expression of PCVAD.

**Conclusion**: Genetic variation among pigs for response to PRRSV and PCV2 exist. Many genes are involved for both viruses. Genetic selection for greater resistance is possible; however, genes involved and an effective selection strategy have not been determined.

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